Attorney Docket No:0003.MSK.P.003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE :	U.S. PATENT NO. 6,028,071
ISSUED:	FEBRUARY 22, 2000
TO:	FRANCIS M. SIROTNAK, et al.
FOR:	PURIFIED COMPOSITIONS OF 10 PROPARGYL-10-DEAZAAMINOPTERIN AND METHODS OF USING SAME IN THE TREATMENT OF TUMORS
FROM:	APPLICATION NO. 09/214,984
371(c) DATE:	MARCH 8, 1999

VIA HAND DELIVERY

MAIL STOP: HATCH-WAXMAN PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AUTHORIZATION TO RELY UPON MARKETING ACTIVITIES IN REQUEST FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. §156

Dear Sir,

Pursuant to M.P.E.P. § 2752, Allos Therapeutics, Inc., a corporation organized and existing under the laws of the State of Delaware, and having a place of business at 11080 CirclePoint Road, Suite 200, Westminster, Colorado 80020, United States of America, hereby authorizes Southern Research Institute, a non-profit corporation organized and existing under the laws of the State of Alabama, and having a place of business at 2000 Ninth Avenue South, Birmingham, Alabama 35255, United States of America; Sloan-Kettering Institute for Cancer Research, a non-profit corporation organized and existing under the laws of the State of New York, and having a place of business at 1275 York

Avenue, New York, New York, 10021, United States of America; and SRI International, a corporation organized and existing under the laws of the State of California, and having a place of business at 333 Ravenswood Avenue, Menlo Park, California 94025, United States of America, to rely upon the activities of Allos Therapeutics, Inc. before the Food and Drug Administration during the regulatory review period for Folotyn™ in its request for extension of patent term under 35 U.S.C. § 156 of U.S. Patent No. Letters Patent of the United States No. 6,028,071 granted to Frances Sirotnak et al. on the 22nd day of February, 2000, for Purified Compositions Of 10 Propargyl-10-Deazaaminopterin And Methods Of Using Same In The Treatment Of Tumors, by virtue of assignments, recorded in the United States Patent and Trademark Office on the 8th day of September, 1997, at Reel 008717, Frame 0125, at Reel 008717, Frame 0128, and at Reel 008719, Frame 0699, subject to an exclusive license granted Allos Therapeutics, Inc.

Allos Therapeutics, Inc. is, and was during the regulatory review period for this product, the licensee of Southern Research Institute, Sloan-Kettering Institute for Cancer Research, and SRI International with respect to this patent as it applies to this product.

Respectfully submitted,
Allos Therapeutics, Inc.

Date: 11/18/09

By: _____

Marc H. Graboyes
Senior Vice President and
General Counsel

S:\ClientFolders\0003 (Allos)\Patent Term Extension PDX\PDX PTE agency letter.DOC

Attorney Docket No:0003.MSK.P.003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE :	U.S. PATENT NO. 6,028,071
ISSUED:	FEBRUARY 22, 2000
TO:	FRANCIS M. SIROTNAK, et al.
FOR:	PURIFIED COMPOSITIONS OF 10 PROPARGYL-10-DEAZAAMINOPTERIN AND METHODS OF USING SAME IN THE TREATMENT OF TUMORS
FROM:	APPLICATION NO. 09/214,984
371(c) DATE:	MARCH 8, 1999

VIA HAND DELIVERY

Mail Stop: Hatch-Waxman PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir,

Transmitted herewith are the application papers of Southern Research Institute, Sloan-Kettering Institute for Cancer Research, and SRI International dated 17 November, 2009 for extension of U.S. Patent No. 6,028,071 under 35 U.S.C. §156, based on the regulatory review period for Folotyn™, together with two duplicate copies as required under 37 C.F.R. § 1.740 (b) and two additional duplicate copies of the application pursuant to M.P.E.P. § 2753, for a total of four copies and one original. Also enclosed pursuant to MPEP § 2572 is a letter from the marketing applicant, Allos Therapeutics, Inc., authorizing Southern Research Institute, Sloan-Kettering Institute for Cancer Research, and SRI International to rely upon the activities of Allos Therapeutics, Inc.

before the Food and Drug Administration during the regulatory review period for Folotyn™ in its request for patent term extension (one original, 4 copies).

As set forth under 37 C.F.R. §1.20(j), included is the sum of $1,120 for the filing of this application for extension of patent term. The undersigned is a registered practitioner of record in the patent and is acting on behalf of the patent owner. See 37 C.F.R. § 1.730(b)(2).

Respectfully submitted,

Date: 17 November 2009

By: Marina I Larson

Marina Larson, #32,038
Attorney for Applicant
Larson & Anderson, LLC
P.O. Box 4928
Dillon, CO 80435-4928

S:\ClientFolders\0003 (Allos)\Patent Term Extension PDX\PDX PTE transmittal.doc

Attorney Docket No:0003.MSK.P.003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE :	U.S. PATENT NO. 6,028,071
ISSUED:	FEBRUARY 22, 2000
TO:	FRANCIS M. SIROTNAK, et al.
FOR:	PURIFIED COMPOSITIONS OF 10 PROPARGYL-10-DEAZAAMINOPTERIN AND METHODS OF USING SAME IN THE TREATMENT OF TUMORS
FROM:	APPLICATION NO. 09/214,984
371(c) DATE:	MARCH 8, 1999

VIA HAND DELIVERY

MAIL STOP: HATCH-WAXMAN PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR EXTENSION OF THE TERM OF UNITED STATES PATENT NO. 6,028,071 UNDER 35 U.S.C. §156

Dear Sir,

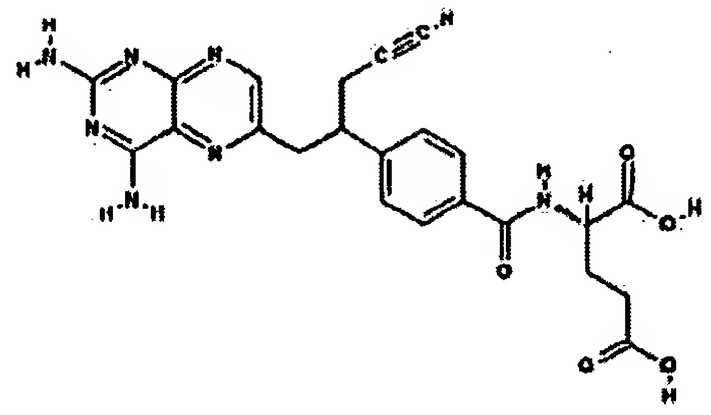
Southern Research Institute, a non-profit corporation organized and existing under the laws of the State of Alabama, and having a place of business at 2000 Ninth Avenue South, Birmingham, Alabama 35255, United States of America; Sloan-Kettering Institute for Cancer Research, a non-profit corporation organized and existing under the laws of the State of New York, and having a place of business at 1275 York Avenue, New York, New York, 10021, United States of America; and SRI International, a corporation organized and existing under the laws of the State of California, and having a place of business at 333 Ravenswood Avenue, Menlo Park, California 94025, United States of America, represents that it is the owner of the entire right, title, and interest in and to

Letters Patent of the United States No. 6,028,071 granted to Frances Sirotnak et al. on the 22nd day of February, 2000, for Purified Compositions Of 10 Propargyl-10-Deazaaminopterin And Methods Of Using Same In The Treatment Of Tumors, subject to an exclusive license granted Allos Therapeutics, Inc., by virtue of assignments, recorded in the United States Patent and Trademark Office (hereinafter referred to as "the Patent Office") on the 8th day of September, 1997, at Reel 008717, Frame 0125, at Reel 008717, Frame 0128, and at Reel 008719, Frame 0699.

Pursuant to the provisions of 37 C.F.R. §1.730, applicant hereby applies for an extension of the term of Patent No. 6,028,071 under 35 U.S.C. §156 until July 16, 2022, which is an extension of 1826 days based on the information set forth herein and in the accompanying papers.

In the materials which follow herein, numbered paragraphs (1) through (15) correspond to paragraphs (1) through (15) of 37 C.F.R. §1.740(a).

(1) The approved product is Folotyn™. Folotyn™ comprises pralatrexate which is represented by the following structural formula:



Chemical name: 10-propargyl-10-deazaaminopterin

USAN Name: (2*S*)-2-[[4-[(1*RS*)-1-[(2,4-diaminopteridin-6-yl)methyl]but-3-ynyl]benzoyl]amino]pentanedioic acid

Generic name: pralatrexate

(2) Folotyn™ was subject to regulatory review under section 505(b) of the Federal Food, Drug and Cosmetic Act, which is codified at 21 U.S.C. §355(b).

(3) Folotyn™ received permission for commercial marketing or use under section 505(b) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. §355(b), on September 24, 2009.

(4) The active ingredient in Folotyn™ is pralatrexate. That active ingredient has not been previously approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act, the Public Health Service Act or the Virus-Serum-Toxin Act.

(5) This application is being submitted within the sixty day period permitted for its submission pursuant to 37 C.F.R. §1.720(f). The last day on which this application could be submitted is November 23, 2009.

(6) The patent for which an extension is being sought is identified as follows:

Inventors: Sirotnak; Francis M. (New York, NY), Piper; James R. (Birmingham, AL), DeGraw; Joseph I. (Missoula, MT), Colwell; William T. (Menlo Park, CA)

Patent No.: 6,028,071

For: Purified Compositions Of 10-Propargyl-10-Deazaaminopterin And Methods Of Using Same In The Treatment Of Tumors

Issued: February 22, 2000

Expires: July 16, 2017

(7) A copy of U.S. Patent No. 6,028,071, the patent for which an extension is being sought, including the entire specification (including claims) and drawings is attached hereto as EXHIBIT A.

(8) Two maintenance fee payments for U.S. Patent No. 6,028,071 have been made to keep the patent in force beyond eight years from its issue date (a copy of the fourth year and eighth year maintenance fee statements are included herewith as EXHIBIT B). A Certificate of Correction was issued on April 8, 2008 (copy attached as Exhibit C). No disclaimers have been made.

(9) (i) Patent No. 6,028,071 claims the approved product.

Claim 1 of Patent No. 6,028,071 reads on Folotyn™ because:

Folotyn™ comprises pralatrexate and claim 1 of Patent No. 6,028,071 recites: "10-Propargyl-10-deazaaminopterin, substantially free of 10-deazaaminopterin."

Claim 2 of Patent No. 6,028,071 reads on Folotyn™ because:

Folotyn™ comprises pralatrexate and claim 2 of Patent No. 6,028,071 recites: "A composition consisting essentially of 10-Propargyl-10-deazaaminopterin."

Claim 3 of Patent No. 6,028,071 reads on Folotyn™ because:

Folotyn™ comprises pralatrexate and a pharmaceutically acceptable carrier and claim 3 of Patent No. 6,028,071 recites: "A pharmaceutical composition comprising 10-Propargyl-10-deazaaminopterin, substantially free of 10-deazaaminopterin, and a pharmaceutically acceptable carrier."

(ii) Patent No. 6,028,071 claims the method of using the approved product.

Claim 4 of Patent No. 6,028,071 reads on the approved method of using Folotyn™ because:

Folotyn™ comprises pralatrexate for the approved use of treatment of human patients with relapsed or refractory peripheral T-cell lymphoma (PTCL) and claim 4 of Patent No. 6,028,071 recites: "A method for treatment of tumors comprising administering to a human patient diagnosed as having a tumor a therapeutically effective amount of 10-propargyl-10-deazaaminopterin, substantially free of 10-deazaaminopterin."

(10) The relevant dates and information pursuant to 35 U.S.C. §156(g) in order to enable the Secretary of Health and Human Services to determine the applicable regulatory review period are as follows.

(i) for a patent claiming a human drug, antibiotic, or human biological product:

A. An exemption under subsection (i) of section 505 of the Federal Food, Drug and Cosmetic Act became effective for Folotyn™ (pralatrexate injection) on March 2, 1997, following receipt by the Food and Drug Administration of Investigational New Drug ("IND") Application No. 52,604 on January 31, 1997.

B. A New Drug Application ("NDA") under section 505(b) of the Federal Food, Drug and Cosmetic Act for Folotyn™ (pralatrexate injection) was initially submitted on March 23, 2009.
This NDA was assigned the number 22-468.

C. NDA No. 22-468 was approved on September 24, 2009.

(11) A brief description of the significant activities undertaken by the marketing applicant during the applicable regulatory review period with respect to the approved product and the significant dates applicable to such activities follows:

The investigational new drug application ("IND") for this drug was submitted on January 31, 1997 and became effective on March 2, 1997. Allos Therapeutics, Inc. has pursued and is pursuing 15 investigations of the use of this drug for the treatment of cancer and related indications. On September 28, 2006, FDA granted fast track designation to the pralatrexate development program for T-cell lymphoma. The NDA was submitted on March 23, 2009. The NDA was approved on September 24, 2009.

The following chart identifies significant communications of substance with the FDA concerning this product:

Date Submitted	**Category**
30-Jan-97	IND filed
31-Jan-97	IND received
05-Feb-97	Confirmation of IND Receipt
06-Feb-97	Response
11-Feb-97	Response
05-Mar-97	Response
07-Mar-97	Response
27-Mar-97	IND Protocol Amend
02-Apr-97	IND CMC Info Amend
02-Apr-97	IND Review Completed
11-Apr-97	IND CMC Info Amend
13-May-97	IND CMC Info Amend
08-Jul-97	IND Protocol Amend
09-Jul-97	Investigator Info
09-Jul-97	IND Protocol Amend

10-Jul-97	IND Protocol Amend
01-Aug-97	IND Protocol Amend
15-Oct-97	Safety Report
16-Oct-97	Safety Report
30-Dec-97	IND CMC Info Amend
22-Jan-98	IND Protocol Amend
04-Jun-98	IND Protocol Amend
12-Aug-98	Annual Report
09-Oct-98	IND Protocol Amend
28-Jun-99	IND Protocol Amend
23-Jul-99	IND Protocol Amend
23-Jul-99	Investigator Info
27-Jul-99	Safety Report
04-Aug-99	IND New Protocol
17-Sep-99	Safety Report
14-Oct-99	Safety Report
28-Oct-99	Annual Report
15-Nov-99	IND New Protocol
02-Mar-00	IND Protocol Amend
03-Apr-00	Safety Report
03-Apr-00	Safety Report
20-Apr-00	Safety Report
01-Jun-00	IND Protocol Amend
30-Jun-00	Safety Report
21-Aug-00	IND Protocol Amend
30-Nov-00	Annual Report
21-Dec-00	IND Protocol Amend
09-Jan-01	Safety Report
12-Jan-01	Safety Report

19-Jan-01	Safety Report
05-Feb-01	Safety Report
19-Mar-01	Safety Report
06-Apr-01	Safety Report
02-May-01	IND New Protocol
03-May-01	IND Protocol Amend
08-Jun-01	IND CMC Info Amend
12-Jun-01	IND PharmTox
15-Jun-01	IND Protocol Amend
25-Jun-01	IND PharmTox
27-Jun-01	Safety Report
17-Jul-01	IND New Protocol
09-Aug-01	Meeting Request
30-Aug-01	Meeting Package
28-Sep-01	IND Protocol Amend
10-Oct-01	Safety Report
19-Nov-01	IND Protocol Amend
07-Dec-01	Safety Report
01-May-02	Safety Report
01-May-02	Safety Report
07-May-02	Change of Chairman
12-Jun-02	Safety Report
13-Jun-02	Annual Report
24-Jun-02	IND Protocol Amend
18-Jul-02	IND Protocol Amend
26-Aug-02	IND Protocol Amend
06-Sep-02	IND New Protocol
16-Sep-02	Safety Report
16-Sep-02	Safety Report

14-Oct-02	IND Protocol Amend
21-Oct-02	Safety Report
31-Oct-02	IND Protocol Amend
19-Nov-02	Safety Report
13-Jan-03	IND Protocol Amend
22-Jan-03	Safety Report
27-Jan-03	Safety Report
30-Jan-03	Transfer letter
03-Feb-03	Transfer letter
26-Feb-03	Acknowledgement of Transfer of IND
04-Mar-03	IND Protocol Amend
06-Mar-03	Safety Report
12-Mar-03	Safety Report
25-Mar-03	IND Protocol Amend
15-Apr-03	Safety Report
30-May-03	IND Protocol Amend
10-Jun-03	Safety Report
17-Jun-03	IND Clin Info Amend
02-Jul-03	Annual Report
27-Aug-03	IND Protocol Amend
02-Sep-03	Safety Report
29-Sep-03	Enrollment on hold
27-Oct-03	IND Protocol Amend
23-Dec-03	Safety Report
16-Jan-04	Safety Report
16-Jan-04	Investigator Info
19-Feb-04	IND Clin Info Amend
27-Apr-04	Safety Report
18-May-04	IND CMC Info Amend

14-Jul-04	IND New Protocol
16-Jul-04	IND PharmTox
29-Jul-04	Annual Report
06-Aug-04	IND Protocol Amend
19-Oct-04	IND Protocol Amend
12-Nov-04	Safety Report
15-Dec-04	Trade Name submission
04-Jan-05	IND Protocol Amend
12-Jan-05	IND Protocol Amend
14-Jan-05	Trade Name amendment
20-Jan-05	Safety Report
09-Mar-05	Trade Name submission
11-Apr-05	IND Protocol Amend
02-May-05	IND Protocol Amend
20-Jul-05	IND PharmTox
29-Jul-05	Annual Report
10-Aug-05	IND PharmTox
17-Aug-05	Trade Name submission
05-Oct-05	IND PharmTox
27-Oct-05	Administrative letter
02-Nov-05	IND PharmTox
07-Nov-05	Safety Report
30-Nov-05	Meeting Request
22-Dec-05	Meeting package
09-Jan-06	IND Protocol Amend
01-Feb-06	IND Protocol Amend
13-Feb-06	Ref. rights letter
16-Feb-06	IND Clin Info Amend
27-Feb-06	Meeting Minutes

01-Mar-06	IND PharmTox
03-Mar-06	IND Clin Info Amend
13-Mar-06	IND New Protocol
16-Mar-06	IND Protocol Amend
03-Apr-06	SPA
07-Apr-06	SPA Questions
18-Apr-06	IND Clin Info Amend
13-Jun-06	IND Protocol Amend
15-Jun-06	IND Protocol Amend
15-Jun-06	Response to SPA
23-Jun-06	IND Protocol Amend
21-Jul-06	Proprietary name submission
26-Jul-06	Annual Report
28-Jul-06	Agreement on SPA
31-Jul-06	Fast Track T cell
04-Aug-06	Investigator Info
08-Aug-06	Safety Report
15-Aug-06	Safety Report
16-Aug-06	Request for Clarification
25-Sep-06	Investigator Info
25-Sep-06	Safety Report
28-Sep-06	Designation of Fast Track
06-Oct-06	IND Protocol Amend
12-Oct-06	Safety Report
20-Oct-06	Safety Report
20-Oct-06	IND Clin Info Amend
26-Oct-06	IND PharmTox
06-Nov-06	Safety Report
30-Nov-06	Investigator Info

30-Nov-06	Safety Report
04-Jan-07	IND Protocol Amend
10-Jan-07	Data disclosure
09-Feb-07	Investigator Info
09-Feb-07	IND New Protocol
13-Feb-07	Safety Report
16-Feb-07	IND Clin Info Amend
27-Feb-07	Safety Report
08-Mar-07	IND Protocol Amend
03-Apr-07	Investigator Info
03-Apr-07	IND New Protocol
05-Apr-07	IND PharmTox
22-May-07	IND Protocol Amend
24-May-07	Investigator Info
24-May-07	Safety Report
24-May-07	IND Protocol Amend
25-May-07	Meeting Request
05-Jun-07	IND PharmTox
06-Jun-07	Safety Report
21-Jun-07	IND Clin Info Amend
21-Jun-07	Withdrawal meeting request
29-Jun-07	Investigator Info
05-Jul-07	Safety Report
16-Jul-07	Safety Report
16-Jul-07	Safety Report
27-Jul-07	Safety Report
27-Jul-07	Safety Report
27-Jul-07	Briefing Package
01-Aug-07	IND Protocol Amend

10-Aug-07	IND New Protocol
14-Aug-07	IND Clin Info Amend
16-Aug-07	Meeting Request
17-Aug-07	Annual Report
24-Aug-07	Safety Report
30-Aug-07	Meeting Request
18-Sep-07	Safety Report
20-Sep-07	SAP
25-Sep-07	Safety Report
28-Sep-07	Briefing Package
01-Oct-07	Briefing Package
15-Oct-07	Investigator Info
18-Oct-07	Safety Report
31-Oct-07	Safety Report
01-Nov-07	IND Protocol Amend
06-Nov-07	Safety Report
13-Nov-07	IND CMC Info Amend
15-Nov-07	IND Protocol Amend
16-Nov-07	IND Protocol Amend
20-Nov-07	IND Protocol Amend
26-Nov-07	IND Clin Info Amend
28-Nov-07	Meeting Request
29-Nov-07	IND Protocol Amend
30-Nov-07	IND Protocol Amend
04-Dec-07	Meeting Minutes
05-Dec-07	Investigator Info
06-Dec-07	IND PharmTox
07-Dec-07	Meeting Request
13-Dec-07	Safety Report

21-Dec-07	Safety Report
10-Jan-08	Briefing Package
10-Jan-08	Briefing Package
15-Jan-08	Meeting Request
24-Jan-08	Safety Report
25-Jan-08	IND Protocol Amend
01-Feb-08	Meeting Minutes
12-Feb-08	Meeting Minutes
20-Feb-08	Briefing Package
21-Feb-08	Safety Report
25-Feb-08	Safety Report
04-Mar-08	Safety Report
05-Mar-08	Safety Report
12-Mar-08	IND Protocol Amend
13-Mar-08	Safety Report
14-Mar-08	IND Protocol Amend
19-Mar-08	Study Plan
25-Mar-08	IND PharmTox
26-Mar-08	Safety Report
27-Mar-08	Safety Report
27-Mar-08	Investigator Info
01-Apr-08	Safety Report
02-Apr-08	Safety Report
02-Apr-08	Meeting Minutes
07-Apr-08	Meeting Request
11-Apr-08	Meeting Request
16-Apr-08	Safety Report
16-Apr-08	SAP
17-Apr-08	IND Clin Info Amend

18-Apr-08	Study Plan
30-Apr-08	IND Clin Info Amend
01-May-08	Safety Report
06-May-08	Briefing package
06-May-08	Charter
09-May-08	Resubmission
12-May-08	IND Protocol Amend
16-May-08	Investigator Info
19-May-08	IND Protocol Amend
21-May-08	Meeting Minutes
28-May-08	Safety Report
10-Jun-08	Investigator Info
11-Jun-08	Briefing package
12-Jun-08	Meeting Request
13-Jun-08	IND PharmTox
27-Jun-08	Meeting Request
30-Jun-08	IND Protocol Amend
07-Jul-08	Response
08-Jul-08	Safety Report
09-Jul-08	Investigator Info
17-Jul-08	Briefing Package
24-Jul-08	Safety Report
25-Jul-08	IND CMC Info Amend
01-Aug-08	Investigator Info
06-Aug-08	SAP
25-Aug-08	Meeting Minutes
26-Aug-08	Safety Report
27-Aug-08	Briefing package
28-Aug-08	IND New Protocol

29-Aug-08	Investigator Info
03-Sep-08	Safety Report
03-Sep-08	Annual Report
08-Sep-08	SAP
09-Sep-08	Briefing package
15-Sep-08	Meeting Minutes
17-Sep-08	Safety Report
18-Sep-08	Safety Report
30-Sep-08	Investigator Info
03-Oct-08	IND Protocol Amend
08-Oct-08	Safety Report
21-Oct-08	Safety Report
22-Oct-08	Responses
29-Oct-08	IND New Protocol
31-Oct-08	Investigator Info
12-Nov-08	IND Protocol Amend
06-Nov-08	Response
24-Nov-08	Investigator Info
26-Nov-08	Safety Report
01-Dec-08	IND New Protocol
04-Dec-08	Safety Report
17-Dec-08	Safety Report
17-Dec-08	Comments
18-Dec-08	Investigator Info
23-Dec-08	Safety Report
08-Jan-09	Safety Report
20-Jan-09	Meeting minutes
23-Jan-09	Investigator Info
26-Jan-09	Safety Report

29-Jan-09	Meeting Minutes
04-Feb-09	Safety Report
06-Feb-09	IND CMC Info Amend
09-Feb-09	Meeting Request
11-Feb-09	Safety Report
13-Feb-09	Safety Report
27-Feb-09	Investigator Info
23-Mar-09	NDA submission 22-468
16-Apr-09	Labeler code
21-Apr-09	NDA Amendment
27-Apr-09	NDA Amendment
29-Apr-09	Labeler code
30-Apr-09	Labeler Code Number Assignment
11-May-09	Acknowledgment of Receipt of NDA on March 23, 2009
12-May-09	NDA Amendment
14-May-09	NDA Amendment
22-May-09	NDA Filed/Prior Review Classification
27-May-09	NDA Amendment
28-May-09	NDA Amendment
01-Jun-09	Request for clinical documentation
03-Jun-09	NDA Amendment
18-Jun-09	NDA Amendment
19-Jun-09	NDA Amendment
29-Jun-09	NDA Amendment
01-Jul-09	NDA Amendment
14-Jul-09	NDA Amendment
21-Jul-09	Information Requests
23-Jul-09	NDA Amendment

30-Jul-09	Briefing package
31-Jul-09	NDA Amendment
31-Jul-09	NDA Amendment
07-Aug-09	Request for CMC
12-Aug-09	Folotyn Acceptable
17-Aug-09	Meeting Minutes
20-Aug-09	NDA Amendment
20-Aug-09	NDA Amendment
21-Aug-09	NDA Amendment
26-Aug-09	NDA Amendment
28-Aug-09	NDA Amendment
01-Sep-09	NDA Amendment
01-Sep-09	Orphan Drug
04-Sep-09	NDA Amendment
09-Sep-09	NDA Amendment
10-Sep-09	NDA Amendment
18-Sep-09	NDA Amendment
18-Sep-09	NDA Amendment
20-Sep-09	NDA Amendment
21-Sep-09	NDA Amendment
21-Sep-09	NDA Amendment
22-Sep-09	NDA Amendment
22-Sep-09	NDA Amendment
22-Sep-09	NDA Amendment
23-Sep-09	DDMAC
24-Sep-09	DDMAC
24-Sep-09	NDA Amendment
24-Sep-09	NDA Accelerated Approval

(12) In the opinion of the Applicant, Patent No. 6,028,071 is eligible for an extension under 35 U.S.C. §156. The length of extension claimed is until July 16, 2022, which is an extension of 1826 days.

The length of extension of term of Patent No. 6,028,071 of until July 16, 2022, which is an extension of 1826 days, claimed by applicant was determined according to the provisions of 37 C.F.R. §1.775 as follows:

According to 37 C.F.R. §1.775(b), the length of extension is equal to the regulatory review period for the approved product, reduced as appropriate pursuant to paragraphs (d)(1) through (d)(6) of 37 C.F.R. §1.775.

According to 37 C.F.R. §1.775(c), the regulatory review period is the sum of: the period of 37 C.F.R. § 1.775(c)(1), which is the number of days in the period beginning on the date the exemption under subsection 505 of the Federal Food, Drug and Cosmetic Act became effective for the approved product and ending on the date the NDA was initially submitted under subsection 505 of the Federal Food, Drug and Cosmetic Act; and the period of 37 C.F.R. § 1.775(c)(2), which is the number of days in the period beginning on the date the NDA was initially submitted under subsection 505 of the Federal Food, Drug and Cosmetic Act and ending on the date the NDA was approved. The exemption under subsection 505(i) of the Federal Food, Drug and Cosmetic Act became effective on March 2, 1997; the date the NDA was initially submitted under subsection 505 of the Federal Food, Drug and Cosmetic Act is March 23, 2009; and the NDA was approved September 24, 2009. Hence the regulatory review period under 37 C.F.R. §1.775(c) is the sum of the period from March 2, 1997 to March 23, 2009 (37 C.F.R. § 1.775(c)(1)) and the period from March 23, 2009 to September 24, 2009 (37 C.F.R. § 1.775(c)(2)). This is the sum of 4405 days and 184 days, which is 4,589 days.

According to 37 C.F.R. §1.775(d)(1)(i), the number of days in the periods of 37 C.F.R. § 1.775(c)(1) and 37 C.F.R. § 1.775(c)(2) which were on and before the date on which the patent issued must be subtracted from the number of days in the regulatory review period. Patent No. 6,028,071 issued on February 22, 2000. The number of days in the period of 37 C.F.R. § 1.775(c)(1) which were on or before February 22, 2000 is calculated as the time period between March 2, 1997 and February 22, 2000, which is

1,088 days. As the period of 37 C.F.R. § 1.775(c)(2) began after the patent issued, no days are subtracted from the period of 37 C.F.R. § 1.775(c)(2). Hence, the number of days to be subtracted under 37 C.F.R. § 1.775(d)(1)(i) is 1,088 days.

37 C.F.R. §1.775(d)(1)(ii) does not apply.

According to 37 C.F.R. §1.775 (d)(1)(iii), from the regulatory review period one then subtracts one-half of the number of days remaining in the period defined in 37 C.F.R. § 1.775(c)(1) after that period is reduced in accordance with 37 C.F.R. § 1.775(d)(1)(i) and 37 C.F.R. § 1.775 (d)(1)(ii). The number of days remaining in the period defined in 37 C.F.R. § 1.775(c)(1) after that period reduced in accordance with 37 C.F.R. § 1.775(d)(1)(i) and 37 C.F.R. § 1.775 (d)(1)(ii) is 4405 days minus 1,088 days which is 3,317 days (37 C.F.R. § 1.775(d)(1)(ii) does not apply). One half of this number of days is 1,658 days.

The calculation is then performed as starting with 4589 days [37 C.F.R. § 1.775 (c)(1) + 37 C.F.R. § 1.775 (c)(2)], subtract 1088 days [37 C.F.R. § 1.775 (d)(1)(i)], then subtract 0 days [37 C.F.R. §1.775(d)(1)(ii)] then subtract 1658 days (37 C.F.R. § 1.775(d)(1)(iii), resulting in 1,843 days.

According to 37 C.F.R. §1.775(d)(2), the reduced regulatory review period of 1,843 days may be added to the expiration date of Patent No. 6,028,071 (July 16, 2017). This gives a date of Tuesday, August 2, 2022.

According to 37 C.F.R. §1.775(d)(3), 14 years may be added to the date of approval of the approved product. This gives a date of September 24, 2023.

According to 37 C.F.R. §1.775(d)(4), the earlier of the dates of 37 C.F.R. §1.775(d)(2) and 37 C.F.R. § 1.775(d)(3) must be selected. The earlier date of these dates is Tuesday, August 2, 2022.

The provisions of 37 C.F.R. §1.775(d)(5) apply to this application because Patent No. 6,028,071 issued after September 24, 1984. Pursuant to 37 C.F.R. §1.775(d)(5)(i), five (5) years are added to the expiration date of Patent No. 6,028,071 (July 16, 2017) giving a date of July 16, 2022. According to 37 C.F.R. §1.775(d)(5)(ii), the dates obtained pursuant to 37 C.F.R. §1.775(d)(5)(i) and 37 C.F.R. §1.775(d)(4) are compared and the earlier date is selected. The date calculated according to 37 C.F.R. §1.775(d)(4)

above is Tuesday, August 2, 2022. Therefore, the earlier of these dates is July 16, 2022. Applicant is entitled to an extension of term of Patent No. 6,028,071 until July 16, 2022.

The provisions of 37 C.F.R. §1.775(d)(6) do not apply because Patent No. 6,028,071 issued on February 22, 2000, which is after September 24, 1984.

(13) Applicant acknowledges a duty to disclose to the Director of United States Patent and Trademark Office and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension which is being sought to the term of Patent No. 6,028,071.

(14) A check for the prescribed fee of $1,120 under 37 C.F.R. §1.20(j) for receiving and acting on this application for patent term extension is enclosed.

(15) Please direct all inquiries and correspondence relating to this application for patent term extension as follows:

Marina Larson

Larson & Anderson, LLC

P.O. Box 4928

Dillon, CO 80435-4928

Pursuant to 37 C.F.R. §1.740(b), two duplicate copies of these application papers are enclosed herewith. Pursuant to M.P.E.P. §2753 an additional two copies of the application are also enclosed herewith. Accordingly, a total of four copies of the application and one original application for patent term extension of Patent No. 6,028,071 are submitted herewith.

Applicant respectfully requests prompt and favorable action on the merits of this application for extension of the term of Letters Patent No. 6,028,071 until July 16, 2022, which is an extension of 1826 days, based on the regulatory review period for Folotyn™. The undersigned is a registered practitioner of record in the patent. See 37 C.F.R. § 1.730(b).

Respectfully submitted,

Date: 17 Nov 2009

Marina Larson, #32,038
Attorney for Applicant
Larson & Anderson, LLC
P.O. Box 4928
Dillon, CO 80435-4928

EXHIBIT A

US006028071A

United States Patent [19]

Sirotnak et al.

[11] Patent Number: 6,028,071

[45] Date of Patent: Feb. 22, 2000

[54] **PURIFIED COMPOSITIONS OF 10-PROPARGYL-10-DEAZAAMINOPTERIN AND METHODS OF USING SAME IN THE TREATMENT OF TUMORS**

[75] Inventors: **Francis M. Sirotnak**, New York, N.Y.; **James R. Piper**, Birmingham, Ala.; **Joseph I. DeGraw**, Missoula, Mont.; **William T. Colwell**, Menlo Park, Calif.

[73] Assignees: **Sloan-Kettering Institute for Cancer Research**, New York, N.Y.; **SRI International**, Menlo Park, Calif.; **Southern Research Institute**, Birmingham, Ala.

[21] Appl. No.: **09/214,984**

[22] PCT Filed: **Jul. 16, 1997**

[86] PCT No.: **PCT/US97/11982**

§ 371 Date: **Mar. 8, 1999**

§ 102(e) Date: **Mar. 8, 1999**

[87] PCT Pub. No.: **WO98/02163**

PCT Pub. Date: **Jan. 22, 1998**

Related U.S. Application Data

[60] Provisional application No. 60/021,908, Jul. 17, 1996.

[51] **Int. Cl.**[7] **A61K 31/505**; C07D 475/08

[52] **U.S. Cl.** **514/249**; 514/258; 544/260; 544/259

[58] **Field of Search** 544/259, 260; 514/249, 258

[56] **References Cited**

U.S. PATENT DOCUMENTS

5,354,751 10/1994 DeGraw et al. 514/249

OTHER PUBLICATIONS

Starling et al., Cancer Chemotherapy Report Part 1, vol. 58., No. 5., Sep./Oct. 1974.

J.I. DeGraw, W.T. Colwell, J.R. Piper, F.M. Sirotnak, "Synthesis and Antitumor Activity of 10–Propargyl–10–deazaaminopterin" *J. Med. Chem.*, 1993, vol. 36, pp. 2228–2231.

Primary Examiner—Mukund J. Shah
Assistant Examiner—Pavanaram K. Sripada
Attorney, Agent, or Firm—Oppedahl & Larson LLP

[57] **ABSTRACT**

Highly purified 10-propargyl-10-deazaaminopterin (10-propargyl-10dAM) compositions tested in xenograft models for their efficacy against human tumors are shown to be far superior to methotrexate ("MTX") and are even superior to the newer clinical candidate edatrexate ("EDX"). Moreover, 10-propragyl-10dAM showed a surprising ability to cure tumors such that there was no evidence of tumor growth several weeks after the cessation of therapy. Thus, highly purified compositions containing 10-propargyl-10dAM can be used to treat human tumors, particularly human mammary tumors and human lung cancer.

12 Claims, 5 Drawing Sheets

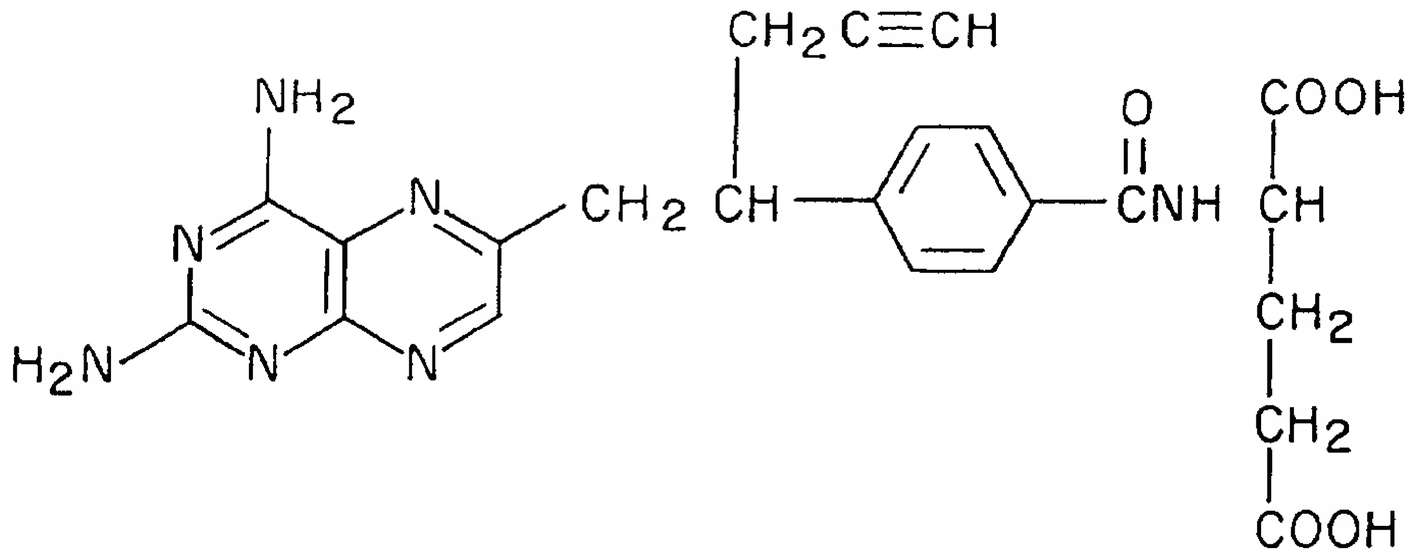


FIG. 1

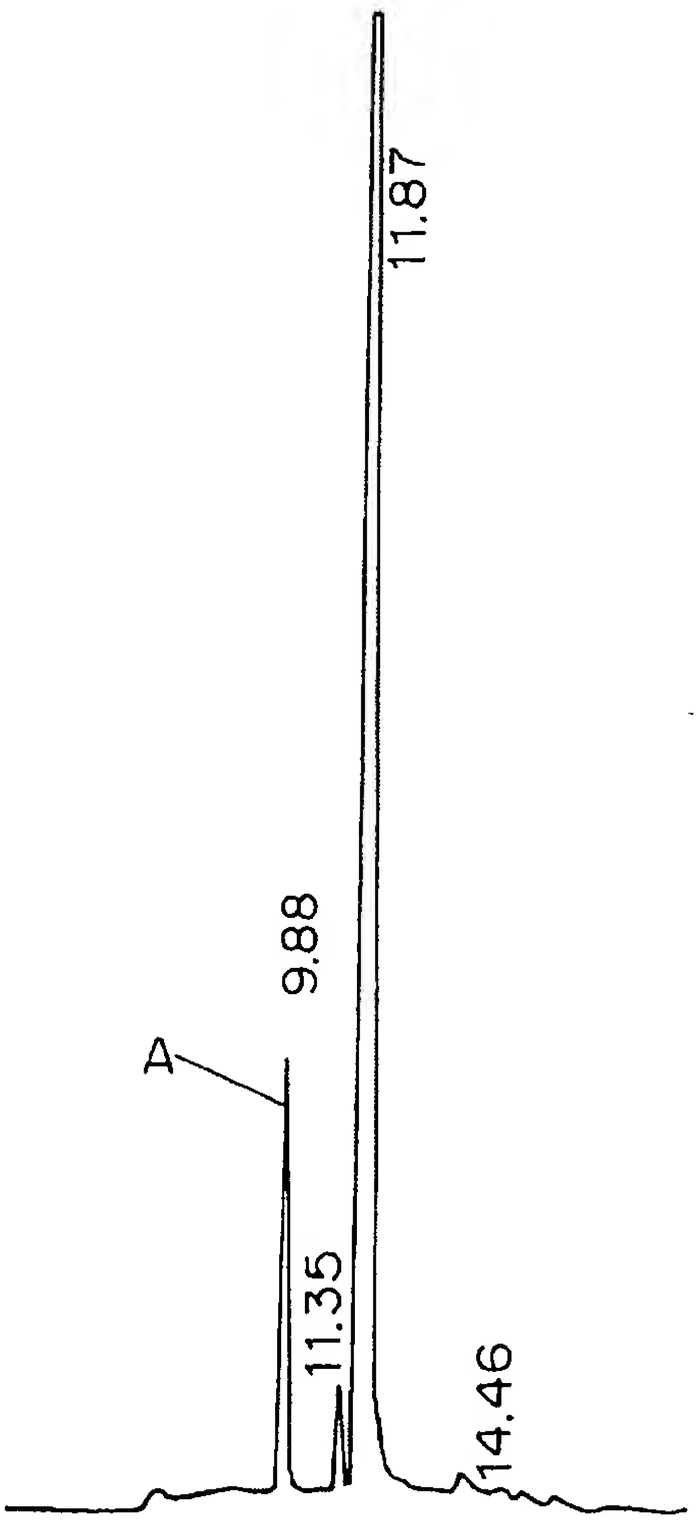


FIG. 2

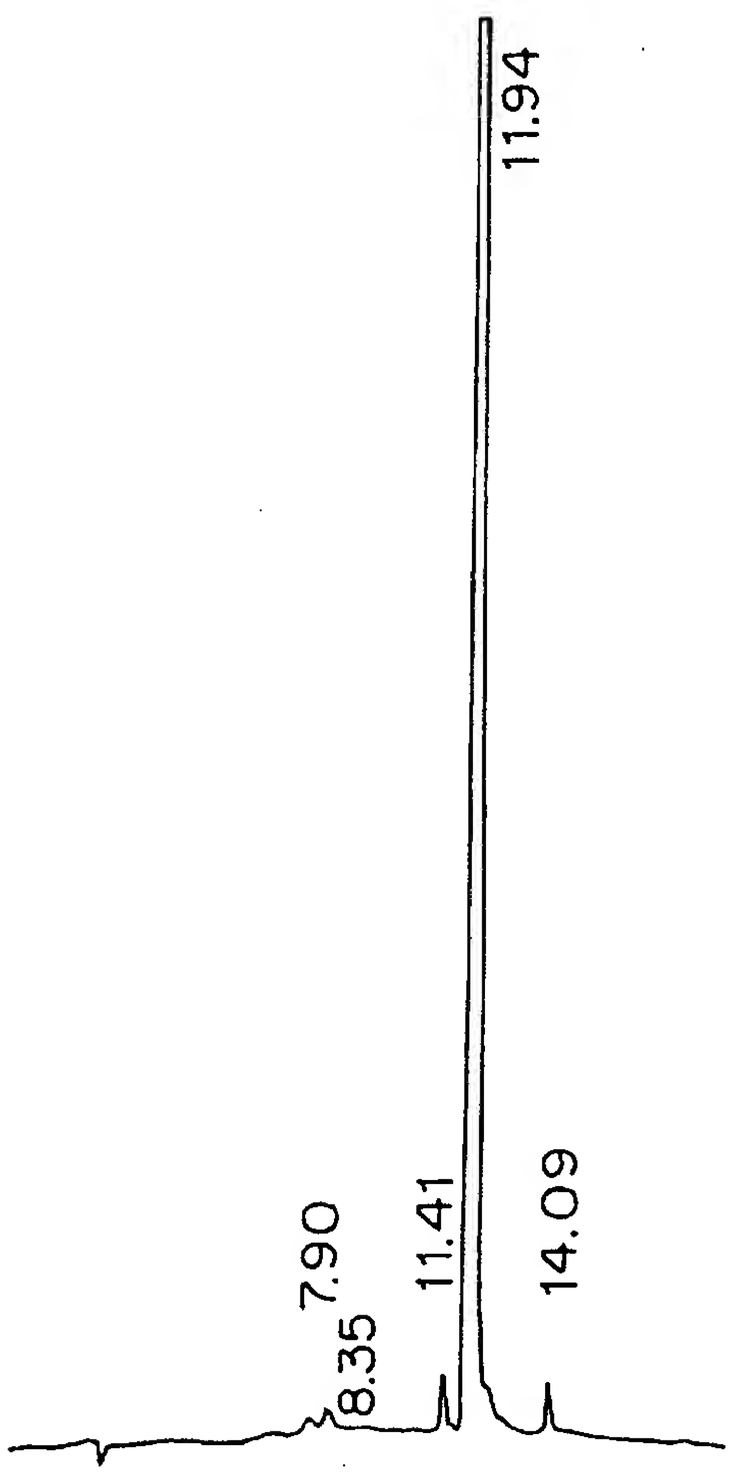


FIG. 3

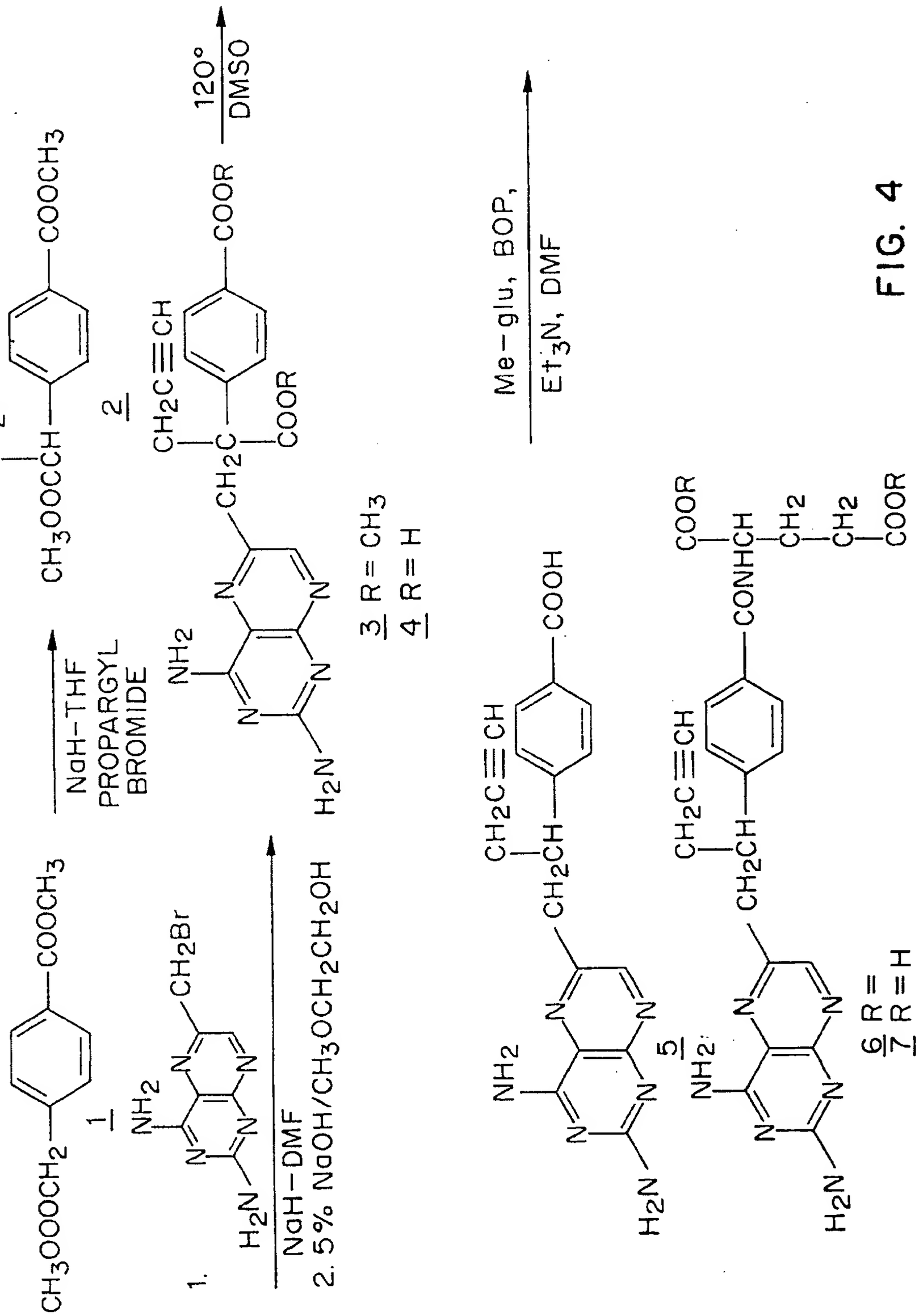

1. NaH—DMF
2. 5% NaOH/CH3OCH2CH2OH
Me—glu, BOP, Et3N, DMF
NaH-THF PROPARGYL BROMIDE
120° DMSO
1
2
3 R = CH3
4 R = H
5
6 R =
7 R = H

FIG. 4

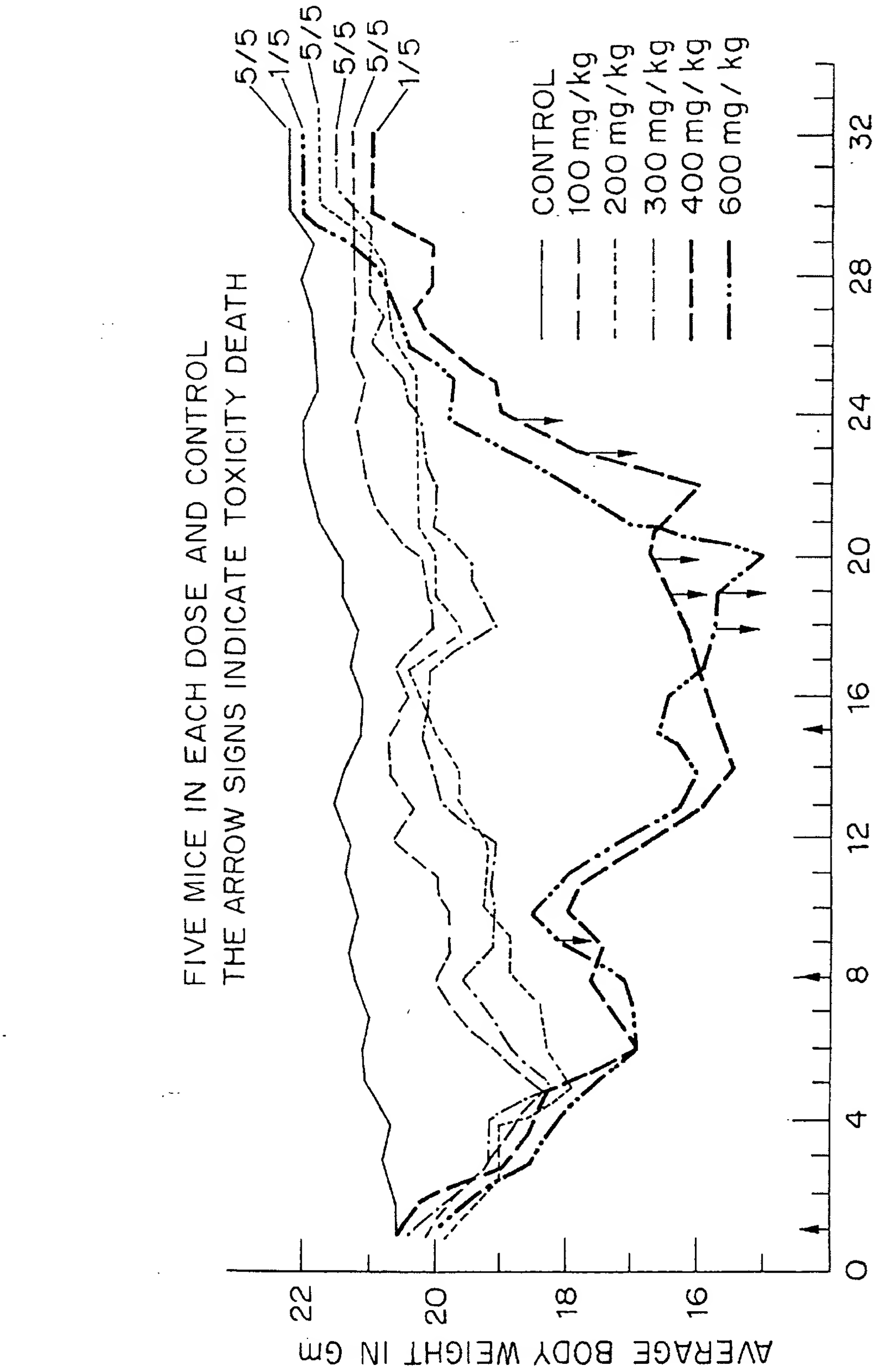

FIVE MICE IN EACH DOSE AND CONTROL
THE ARROW SIGNS INDICATE TOXICITY DEATH
5/5
1/5
5/5
5/5
5/5
1/5
CONTROL
100 mg/kg
200 mg/kg
300 mg/kg
400 mg/kg
600 mg/kg
AVERAGE BODY WEIGHT IN Gm
22
20
18
16
0
4
8
12
16
20
24
28
32
DAYS AFTER START OF THE TREATMENT

FIG. 5

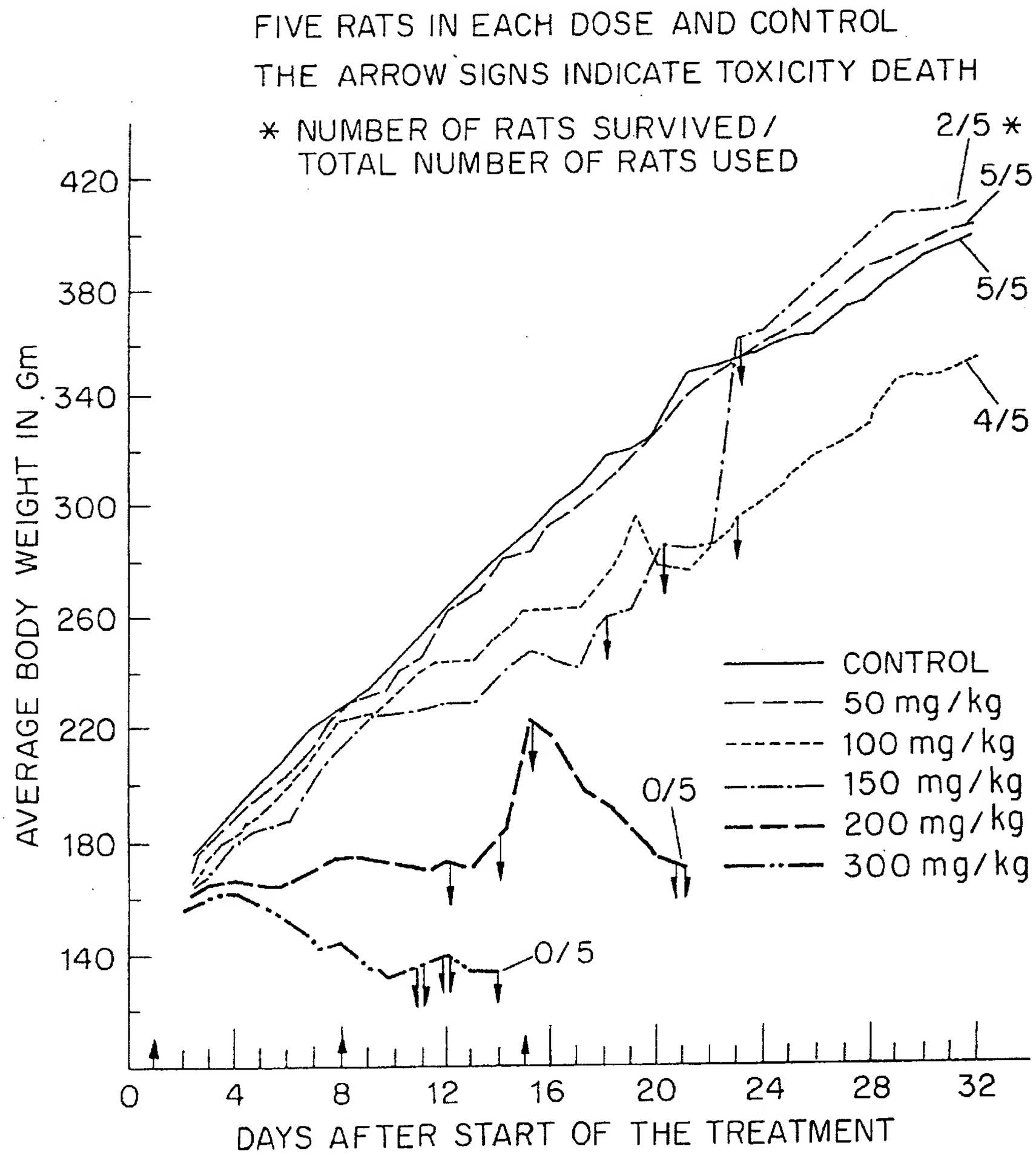

FIVE RATS IN EACH DOSE AND CONTROL
THE ARROW SIGNS INDICATE TOXICITY DEATH
* NUMBER OF RATS SURVIVED / TOTAL NUMBER OF RATS USED
AVERAGE BODY WEIGHT IN Gm
420
380
340
300
260
220
180
140
0
4
8
12
16
20
24
28
32
DAYS AFTER START OF THE TREATMENT
2/5 *
5/5
4/5
0/5
0/5
CONTROL
50 mg/kg
100 mg/kg
150 mg/kg
200 mg/kg
300 mg/kg

FIG. 6

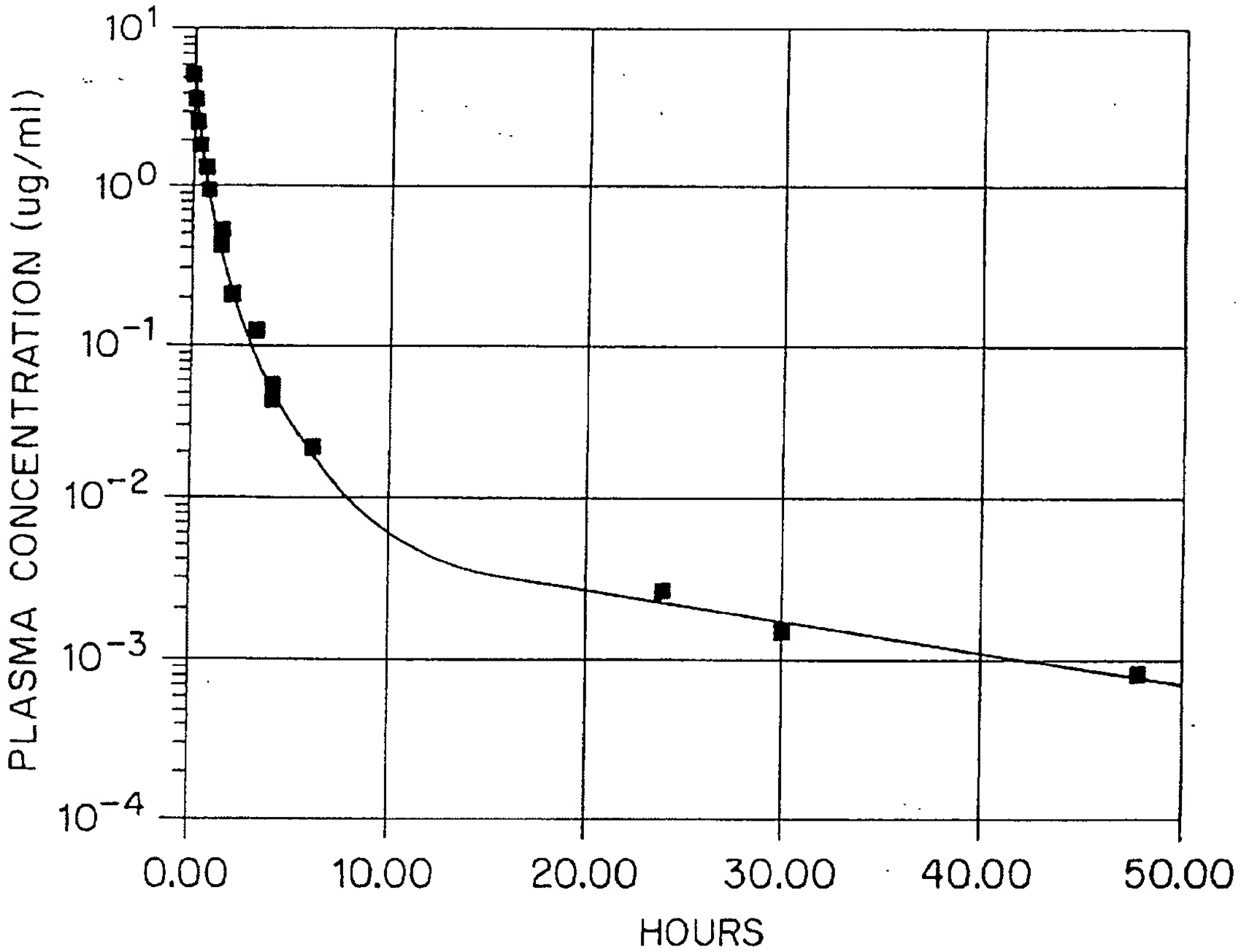

PLASMA CONCENTRATION (ug/ml)
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
0.00
10.00
20.00
30.00
40.00
50.00
HOURS

FIG. 7

PURIFIED COMPOSITIONS OF 10-PROPARGYL-10-DEAZAAMINOPTERIN AND METHODS OF USING SAME IN THE TREATMENT OF TUMORS

This application is based on provisional appln 60/021,908 filed Jul. 17, 1996 and is 371 of PCT/US97/11982 filed Jul. 16, 1997.

BACKGROUND OF THE INVENTION

This application relates to a purified composition of the compound 10-propargyl-10-deazaaminopterin and to methods of using this compound in the treatment of tumors.

10-Propargyl-10-deazaaminopterin ("10-propargyl-10dAM") is a member of a large class of compounds which have been tested and in some cases found useful in the treatment of tumors. This compound, which has the structure shown in FIG. 1, was disclosed by DeGraw et al., "Synthesis and Antitumor Activity of 10-Propargyl-10-deazaaminopterin," *J. Medical Chem.* 36: 2228–2231 (1993) and shown to act as an inhibitor of growth in the murine L1210 cell line and to a lesser extent of the enzyme dihydrofolate reductase ("DHFR"). In addition, some results were presented for the antitumor properties of the compound using the E0771 murine mammary tumor model. This data was equivocal because of the small number of mice used in the test (3 per dosage), the absence of any standard deviation information which would quantify the reliability of the data, and the fact that the highest dose used was in fact toxic to the mice. Nevertheless, assuming this data has some predictive value for the efficacy of a drug in treating human tumors, it would at best predict a drug which, at equivalent levels of tolerance, had properties comparable to or perhaps slightly better than methotrexate.

SUMMARY OF THE INVENTION

Surprisingly, however, more highly purified 10-propargyl-10dAM compositions when tested in a xenograft model for their efficacy against human tumors have now been shown to be far superior to methotrexate ("MTX") and are even superior to edatrexate ("ETX"), a more recent clinical candidate. Moreover, 10-propargyl-10dAM showed a surprising ability to cure tumors such that there was no evidence of tumor growth several weeks after the cessation of therapy. Thus, a first aspect of the present invention is a highly purified composition containing 10 -propargyl-10dAM. This composition can be used in accordance with the invention to treat tumors, particularly human mammary tumors and human lung cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the structure of 10-propargyl-10dAM;

FIG. 2 shows an HPLC of an impure 10-propargyl-10dAM preparation prepared in accordance with the prior art;

FIG. 3 shows an HPLC of a highly purified 10-propargyl-10dAM preparation in accordance with the invention;

FIG. 4 shows a synthetic scheme useful in preparing the compound in accordance with the invention;

FIG. 5 summarizes the results of toxicity testing in mice;

FIG. 6 summarizes the results of toxicity testing in rats; and

FIG. 7 shows average plasma concentrations after administration of 10-propargyl-10dAM in dogs.

DETAILED DESCRIPTION OF THE INVENTION

This application relates to "highly purified" 10-propargyl-10dAM. As used in the specification and claims hereof, compositions which are "highly purified" contain 10-propargyl-10dAM substantially free of other folic acid derivatives particularly 10-deazaaminopterin, which can interfere with the antitumor activity of the 10-propargyl-10dAM. A composition within the scope of the invention may include carriers or excipients for formulating the 10-propargyl-10dAM into a suitable dosage unit form for therapeutic use.

10-propargyl-10dAM can be synthesized using the method disclosed in the DeGraw paper, supra or in Example 7 of U.S. Pat. No. 5,354,751 which is incorporated herein by reference. HPLC evaluation of the product prepared by this method shows the presence of a substantial amount (~4.6%) of an impurity A (FIG. 2) which has a retention time consistent with 10-deazaaminopterin. Thus, if this synthetic approach is employed further purification is necessary beyond that disclosed in the DeGraw et al. paper. Such purification can be carried out by additional HPLC or crystallization to remove the 10-deazaaminopterin and other folic acid derivatives which may be present.

FIG. 3 shows an HPLC of a highly purified preparation consisting essentially of 10-propargyl-10dAM in accordance with the invention prepared using the method described in Example 1. In this case, the amount of 10-propargyl-10dAM (as determined by HPLC peak area) approaches 98%, and the peak corresponding to 10-deazaaminopterin is not detected by the processing software although there is a minor baseline ripple in this area.

The highly purified 10-propargyl-10dAM preparation in accordance with the invention was tested for cytotoxicity against human tumor cell lines and antitumor properties using xenografts of human tumor lines in nude mice as described in Example 2. The results of these tests are summarized in Tables 1 and 2. As shown, 10-propargyl-10dAM effected complete regressions of human MX-1 mammary carcinoma to a far greater extent than either MTX (which caused no regressions) or EDX, and was in fact able to effect cures in 9 out of the 20 mice tested. 10-propargyl-10dAM was also far more effective than MTX and EDX against xenografts of human LX-1 lung cancer and led to cures in 4 of the 10 mice tested. Similar results were observed for human A549 lung cancer cells. This level of efficacy is far in excess of anything which could have been predicted based upon the E0771 data which appeared in the DeGraw et al. paper. In fact, in that study no mice treated with the lower, non-toxic dosage level (24 mg/kg) of 10-propargyl-10dAM showed complete regression of the tumors and the average effect of the compound was no better than MTX. These 10-P-dAM treated mice showed an increase in tumor size at the end of three weeks, indicating that a cure had not been effected. It is therefore very surprising that the highly purified compound can be used against human tumors at much lower dosage levels (3 mg/kg) and achieve much higher levels of efficacy and many apparent cures.

While not intending to be bound by any particular mechanism for this increase in activity, it is believed that the presence of even relatively small amounts of other folic acid derivatives such as the 4.6% 10-deazaaminopterin observed in the samples prepared in the DeGraw et al paper can compete with the 10-propargyl-10dAM. effectively inhibiting its activity. This could happen at the level of poly-

glutamylation of 10-propargyl-10dAM by folyl polyglutamate synthetase in human tumor cells. The advantage of 10-propargyl-10dAM as substrate for this cytotoxic determinant could be compromised by the presence of 10-dAM which more effectively interacts with this enzyme, but it poorly metabolized thus competitively inhibiting the interaction of 10-propargyl-10dAM with that enzyme. Regardless of the mechanism, however, the highly purified compositions of the invention are markedly more active against human cancer cells than would be predicted based upon the data presented in the DeGraw paper. This is also shown by the increased cytotoxicity of 10-propargyl-10dAM compared to EDX against human tumor cells that was consistently found, and which contrasts with the relative equivalence of these two compounds against murine tumor cells lines as reported by DeGraw et al. This enhanced activity against human tumor cells can be used to provide therapeutic benefits to human patients suffering from cancer, particularly from breast cancer or lung cancer.

For this purpose, the highly purified 10-propargyl-10dAM is advantageously formulated as part of a pharmaceutical preparation. The specific dosage form will depend on the method of administration, but may include tablets, capsules, oral liquids, and injectable solutions for intravenous, intramuscular or intraperitoneal administration. Based upon the relative effectiveness of MTX, EDX and 10-Propargyl-10-deazaaminopterin, substantially free of 10-deazaaminopterin against human xenograft tumors, and on the dosages of MTX and EDX found to be appropriate in human clinical trials, dosages of 10-Propargyl-10-deazaaminopterin, substantially free of 10-deazaaminopterin in the range of from 40 to 120 mg/m^2 of body surface area/day should be effective, depending on the treatment schedule. Higher doses would appear to be contraindicated because of the toxicity observed at such levels in animal studies reported below.

10-Propargyl-10-dAM in accordance with the invention may also be formulated in combination with a variety of other cytotoxic and antitumor compounds, including vinca alkaloids such as vinblastine, navelbine and vindesine; 5-fluorouracil; alkylating agents such as cyclophosphamide or ifosfamide: cisplatin or carboplatin; leucovorin; taxols such a paclitaxel or docetaxel; and antibiotics such as doxorubicin and mitomycin. Combinations of 10-propargyl-10dAM with several of these other antitumor agents may also be used.

EXAMPLE 1

FIG. 4 shows a synthetic scheme useful in preparing 10-propargyl-10-dAM in accordance with the invention. A mixture of 60% NaH in oil dispersion (1.06 g, 26.5 mmol) in 18 mL of sieve-dried THF was cooled to 0° C. The cold mixture was treated with a solution of homoterephthalic acid dimethyl ester (5.0 g, 24 mmol. compound **1** in FIG. **4**) in dry THF (7 mL), and the mixture was stirred for 1 hour at 0° C. Propargyl bromide (26.4 mmol) was added, and the mixture was stirred at 0° C. for an additional 1 hour, and then at room temperature for 16 hours. The resulting mixture was treated with 2.4 mL of 50% acetic acid and then poured into 240 mL of water. The mixture was extracted with ether (2×150 mL). The ether extracts were combined, dried over Na_2SO_4, and concentrated to an orange-yellow oil. Chromatography on silica gel (600 mL of 230–400 mesh) with elution by cyclohexane-EtOAc (8:1) gave the product α-propargylhomoterephthalic acid dimethyl ester (compound **2**) as a white solid (4.66) which appeared by TLC (cyclohexane-EtOAc, 3:1) to be homogeneous. Mass spectral data on this product, however, showed it to be a mixture of the desired product **2**, and the dipropargylated compound. No starting material **1** was detected. HPLC shows the ratio of mono- to di-propargylated products to be about 3:1. Since the dipropargylated product, unlike compound **1**, cannot produce an unwanted coproduct in the next step of the reaction, this material was suitable for conversion to compound **3**. Absence of starting compound **1** in the product used to proceed in the synthesis is very important in order to avoid the sequential formation of 10-dAM during the transformations lading to the final product, because complete removal from 10-dAM from 10-propargyl-1-dAM is very difficult.

A mixture was formed by combining 0.36 g of a 60% NaH (9 mmol) in oil dispersion with 10 mL of dry DMF and cooled to 0–5° C. The cold mixture was treated drop-wise with a solution of the product of the first reaction (compound **2**) (2.94 g, 12 mmol) in 10 mL dry DMF and then stirred at 0° C. for 30 minutes. After cooling to −25° C., a solution of 2,4,diamino-6-(bromomethyl)pteridine hydrobromide-0.2 2-propanol (1.00 g, 2.9 mmol) in 10 mL dry DMF was added drop-wise while the temperature was maintained near −25° C. The temperature of the stirred mixture was allowed to rise to −10° C. over a period of 2 hours. After an additional 2 hours at −10° C., the temperature was allowed to rise to 20° C., stirring at room temperature was continued for 2 hours longer. The reaction was then adjusted to pH 7 by addition of solid CO_2, After concentration in vacuo to remove solvent, the residue was stirred with diethyl ether and the ether insoluble material was collected, washed with water, and dried in vacuo to give 1.49 g of a crude product. This crude product was dissolved in $CHCl_3$—MeOH (10:1) for application to a silica gel column. Elution by the same solvent system afforded 10-propargyl-10-carbomethoxy-4-deoxy-4-amino-10-deazapteroic acid methyl ester (compound **3**) which was homogenous to TLC in 40% yield (485 mg).

A stirred suspension of compound **3** (400 mg, 0.95 mmol) in 2-methoxycthanol (5 mL) was treated with water (5 mL) and then 10% sodium hydroxide solution (3.9 mL). The mixture was stirred as room temperature for 4 hours, during which time solution occurred. The solution was adjusted to pH 8 with acetic acid and concentrated under high vacuum. The resulting residue was dissolved in 15 mL of water and acidified to pH 5.5–5.8 resulting in formation of a precipitate. The precipitate was collected, washed with water and dried in vacuo to recover 340 mg of compound **4** (91% yield). HPLC analysis indicated a product purity of 90%.

Compound **4** (330 mg) was decarboxylated by heating in 15 mL DMSO at 115–120° C. for 10 minutes. A test by HPLC after 10 minutes confirmed that the conversion was essentially complete. DMSO was removed by distillation in vacuo (bath at 40° C.). The residue was stirred with 0.5 N NaOH to give a clear solution, Acidification to pH 5.0 with 1 N HCl gave 10-propargyl-4-deoxy-4-amino-10-deazapteroic acid (compound **5**) as a yellow solid in 70% yield. HPLC indicated product purity at this stage as 90%.

Compound **5** (225 mg, 0.65 mmol) was coupled with dimethyl L-glutamate hydrochloride (137 mg, 0.65 mmol) using BOP reagent (benzotriazole-1-yloxytris (dimethylamino) phosphonium hexafluorophosphate (287 mg, 0.65 mmol, Aldrich Chemical Co.) in DMF (10 mL) containing triethylamine (148 mg, 1.46 mmol). The mixture was stirred for 3 hours at 20–25° C. and then evaporated to dryness. The residue was stirred with water, and the water-insoluble crude product was collected and dried in vacuo. The crude product (350 mg) was purified by silica gel

chromatography with elution by $CHCl_3$—MeOH (10:1) containing triethylamine (0.25% by volume) to recover 165 mg of 10-propargyl-10-deazaaminopterin dimethyl ester (compound **6**, 50% yield) which was homogeneous to TLC ($CHCl_3$—MeOH 5:1).

Compound **6** (165 mg, 0.326 mmol) was suspended in 10 mL stirred MeOH to which 0.72 mL (0.72 meq) 1 N NaOH was added. Stirring at room temperature was continued until solution occurred after a few hours. The solution was kept at 20–25° C. for 8 hours, then diluted with 10 mL water. Evaporation under reduced pressure removed the methanol, and the concentrated aqueous solution was left at 20–25° C. for another 24 hours. HPLC then showed the ester hydrolysis to be complete. The clear aqueous solution was acidified with acetic acid to pH 4.0 to precipitate 10-propargyl-10-deazaaminopterin as a pale yellow solid, The collected, water washed and dried in vacuo product weighed 122 mg (79% yield). Assay by elemental analysis, proton NMR and mass spectroscopy were entirely consistent with the assigned structure. HPLC analysis indicated purity of 98% and established the product to be free of 10-deazaaminopterin.

EXAMPLE 2

The highly purified 10-propargyl-10dAM preparation prepared in accordance with Example 1 was tested for antitumor properties using xenografts of human tumor lines in nude mice. Xenografts of human MX-1 mammary carcinoma were implanted into nude mice by standard procedures.

To test the antitumor properties of 10-propargyl-10dAM against these tumor cells, 3 mg/kg of the compound was administered once a day to each of twenty mice for a total of five days starting three days after tumor implantation. For comparison, untreated controls (20 mice), methotrexate treated mice (10 mice; dosage 2 mg/kg on the same treatment schedule) and edatrexate treated mice (20 mice; dosage 1.5 mg/kg on the same treatment schedule) were also evaluated. These doses are all "maximum tolerated doses" and thus are an appropriate basis for comparison based upon equitoxicity. Average tumor diameter was measured 14 days after the start of treatment, i.e., 7 days after the cessation of treatment. Mice which had no measurable tumor at this time were considered to have undergone a complete regression. In addition, mice which were tumor free at 14 days were checked three weeks after cessation of therapy for the reappearance of tumors. Tumor free mice at the end of three weeks after therapy were considered to be cured. The results are summarized in Table 1.

TABLE 1

Treatment	Average Tumor Diameter (mm)	Complete Regressions	Cures
untreated	8.6 ± 0.9	0/20	0/20
MTX	7.6 ± 0.8	0/10	0/10
EDX	2.2 ± 1.1	6/20	2/20
10-propargyl-10 dAM	0.3	13/20	9/20

As can be seen, 10-propargyl-10dAM is substantially more effective than either MTX or EDX, and effected a substantial number of cures.

EXAMPLE 3

Example 2 was repeated using xenografts of human LX-1 lung cancer in nude mice. The results are summarized in Table 2.

TABLE 2

Treatment	Average Tumor Diameter (mm)	Complete Regressions	Cures
untreated	10.2 ± 1.8	0/10	0/10
MTX	9.2 ± 2	0/10	0/10
EDX	4.3 ± 2	3/10	1/10
10-propargyl-10 dAM	0.4	9/10	4/10

Again, 10-propargyl-10dAM was shown to be substantially more effective than MTX or EDX, and effected a substantial number of cures.

EXAMPLE 4

Example 2 was repeated using xenografts of human A549 lung cancer in nude mice. The results are summarized in Table 3.

TABLE 3

Treatment	Average Tumor Diameter (mm)	Complete Regressions	Cures
untreated	8.9 ± 1	0/5	0/5
MTX	8.3 ± 2	0/5	0/5
EDX	6.8 ± 2	0/5	0/5
10-propargyl-10 dAM	4.2 ± 2	3/10	2/10

Again, 10-propargyl-10dAM was shown to be substantially more effective than MTX or EDX, and effected a substantial number of cures.

EXAMPLE 5

Cytotoxicity studies were performed on four human tumor cells lines to compare the cytotoxicity of EDX to 10-propargyl-10dAM using a 3 hour pulse-exposure to each compound. Three replicate experiments of each cell line were tested for each compound. The results are summarized in Table 4.

TABLE 4

Tumor	Tissue Type	IC_{50} - EDX	IC_{50} 10-propargyl-10 dAM
MDA468	lung	0.38 ± 0.05	0.11 ± 0.01
SKLC-16	lung	0.26 ± 0.03	0.10 ± 0.014
ZR-75-1	mammary	0.86 ± 0.1	0.28 ± 0.05
SK-BRIII	mammary	0.99 ± 0.15	0.14 ± 0.02

In each case, the 10-propargyl-10dAM was substantially more cytotoxic than EDX against the human tumor cells lines.

EXAMPLE 6

Toxicity of 10-propargyl-10dAM was assessed in rats, mice and dogs. Male CD rats and male $B6D2F_1$ mice (Charles River Breeding Laboratories, Wilmington, Mass.) and young adult male beagle dogs (Marshall Frams USA. Inc., Northrose, N.Y.) were used in the tests. All animals were maintained in environmentally controlled rooms with a 12 hours light/12 hour dark light cycle. Mice and rats were received when 5 weeks old and were observed for 1 to 2 weeks before study and used only if their growth during the preliminary observation matched laboratory standards for weight-gain. Dogs were observed at least 2–3 weeks before

use, during which period they were weighted and examined at regular intervals to assure good health. During the test period, all animals were weighted daily and observed for appetite, stool conditions, general appearance and signs of toxicity. Dogs were also examined daily to monitor body temperature, heart rate, and respiration rate.

For all treatments, the dose of drug was weighed and dissolved in isotonic bacteriostatic saline by addition of about 2 molar equivalents of 1 N NaOH. The pH of this solution was adjusted to 7–7.2 by addition of NaOH solution as determined using a pH meter. Solutions were used either immediately or after thawing preparations that had been stored at -20° C. Injections in mice and rats were made in a constant volume of 0.01 ml/g of body weight.

Toxicity in Mice

$B6D2F_1$ mice, five per group, were given 10-propargyl-10dAM i.p. weekly for three weeks (days 1, 8 and 15) at varying concentrations as summarized in Table 5.

TABLE 5

Treatment Level	Survivors After 32 days
control	5/5
100 mg/kg	5/5
200 mg/kg	5/5
300 mg/kg	5/5
400 mg/kg	1/5
600 mg/kg	1/5

The results of body weight changes and lethality are summarized in FIG. 5. As shown, at 100, 200 and 300 mg/kg there were initial moderate declines in body weight (up to 2 grams), but no further drops in the subsequent doses. All mice in these three dosage groups regained weight in weeks 3 and 4 and survived. At dosages of 400 mg/kg, i.p. QWX3, four out of five mice died on days 19, 20, 23 and 24, and a 600 mg/kg, four out of five mice died on days 9, 18, 19 and 21. Those animals treated with the higher two doses had more than 20% weight loss, ruffled fur and diarrhea. However, surviving mice gained weight and caught up with the control group two weeks after the final injections. The approximate $LD_{_}$was about 370 mg/kg. i.p. QWX3 when estimated with dose effect relationship and the median-effect plot. (Chou et al, *Encyclopedia of Human Biology,* R. Dalbecco, ed., Vol. 2, pp. 271–279, Academic Press, 1991.)

Toxicity in Rats

CD rats, five per group, were given 10-propargyl-10dAM i.v. weekly for three weeks (days 1, 8 and 15) at varying concentrations as summarized in Table 6.

TABLE 5

Treatment Level	Survivors After 32 days
control	5/5
50 mg/kg	5/5
100 mg/kg	4/5
150 mg/kg	2/5
200 mg/kg	0/5
300 mg/kg	0/5

The results of body weight changes and lethality are summarized in FIG. 6. At 50 mg/kg, i.v. QWX3, no apparent changes in body weight were observed; at 100 mg/kg, there were approximately 10 gram decreases in body weight at the third dose, and one out of the five animals had died by day 23. The remaining animals gained weight, but at a rate less than that of control animals.

At 150 mg/kg, i.v. QWX3, three out of the five rats died on days 18, 20, and 23. At 200 mg/kg, i.v. QWX3 all five rats died on days 12, 14, 15, 20 and 20, respectively. At 300 mg/kg, i.v. QWX3 all five rats also died, but somewhat sooner that those on the 200 mg/kg dosage, on days 11, 11, 12, 12, and 14, respectively. No immediate toxicity was observed in any of the rats immediately after injection at the 150–300 mg/kg dosages. These rats began to lose weight the following day, and had ruffled fur with evidence of diarrhea and dehydration which culminated one to three days before death. These signs persisted through the course of the experiment and were exacerbated by the second and third injections.

The data from these experiments did not allow a precise calculation of LD_{10} or LD_{50}. A conservative estimate of LD_{10} in rats with the dose-effect relationship and the median-effect plot is about 75 mg/kg, i.v. QWX3 and LD_{50} is about 110 mg/kg i.v. QWX3.

Toxicity in Dogs

Eight male beagles weighing 9.4 to 10.6 pounds were divided into four pairs. The pairs were treated with intravenous injections of 10-propargyl-10dAM weekly for three weeks (days 1, 8 and 15) at 0 mg/kg (dogs A and B); 3 mg/kg (dogs C and D); 8 mg/kg (dogs E and F) and 12 mg/kg (dogs G and H). At 3 mg/kg and 8 mg/kg, a maximal body weight decrease of 2 to 3 kg occurred on day 20. followed by body weight recovery thereafter through the end of the 35 day observation period. At 12 mg/kg there were steady declines in body weight totaling up to 3 kg (or more than 20% loss), and the animals became moribund on day 12 and 14, prior to the third dosage.

Major signs of toxicity were observed for the dogs treated at 8 mg/kg or 12 mg/kg, including vomiting, diarrhea, watery or bloody stool, lethargy, anoreptic, and generalized weakness. At 3 mg/kg, i.v. QWX3, no symptoms were apparent. The estimated LD_{50} is about 8 mg/kg, i.v., QWX3.

Blood samples were drawn from each of the dogs during the testing. No marked or persistent change sin blood chemistry or blood cell counts were observed, except at terminal phases of toxicity. There were some declines in white blood cells, lymphocytes, neutrophil counts, decreases in hemoglobin, total protein and albumin, and increases in amylase and monocytes were observed, especially at the higher two doses.

Dogs G and H were euthanized and a complete necropsy was performed on days 12 and 14, respectively. One animal from each of the other pairs were euthanized for histopathological examination on day 33 (dog E) or 34 (dogs B and C) of the experiment.

In Dog G, most of the organs appeared normal. The mucosa of small and large intestines showed edema and hemorrhagic. Stomach and the large and small intestines were empty. Dog H was severely depressed, with shallow breathing and reduced heart rate on day 14 prior to euthanasia. Upon euthanasia, the stomach was found to be filled with bile-tinged mucous, and the intestines were filled with watery stool but no signs of blood. No ulcers in the stomach, intestine or esophagus were observed. The liver was pale and spotty. The spleen was dark purple and rough on the surface.

For dog E which received 8 mg/kg, i.v. QWX3, most organs appeared normal but both sides of the lung were pink with a few bloody spots. Large and small intestines and liver

showed lavender color and kidneys showed edema and purple color. Stomach and intestines were full with food, and bladder full with urine.

Dog C, which received 3 mg/kg, i.v. QWX3, appeared normal on day 34 prior to euthanasia. Most organs appeared normal. The right lung was pink, large and small intestines purple in color. Liver showed dark purple color. Stomach and intestines were full with food, and bladder full with urine.

Histopathological examination of tissues of dogs treated with 0 mg/kg and 3 mg/kg i.v., QWX3, showed no significant lesions in the organ specimens collected. At 8 mg/kg, i.v. QWX3, the large intestines and multicocal mild colitis. At 12 mg/kg, i.v. QWX3, subacute to chronic ulcerative esophagitis and severe necrotizing enterocolitis were observed. Other organs, e.g., brain, heart, liver, lung, kidney, salivary gland, testis and spleen showed no significant lesions.

EXAMPLE 7

To monitor the pharmacokinetics of the 10-propargyl-10dAM, single doses of 3 mg/kg were given intravenously to each of two dogs, I and J. Blood samples were collected at −5 min, 5 min, 10 min, 20 min, 30 min, 45 min, 60 min, 90 min, 3 hr, 4 hr, 6 hr, 24 hr, 30 hr and 48 hr. 10-propargyl-10dAM concentrations in plasma were determined by a fluorometric high performance liquid chromatography (HPLC) method using an Econosphere C18 column, 15% acetonitrile/KH_2PO_4 50 mm mobile phase, pH 7.0, with a 1 ml/min flow rate at room temperature. The injection volume was 1 ul. The retention time of 10-propargyl-10dAM was 18.5 minutes.

The plasma half-life ($t_{1/2}$) for dog I were 26.7 min. 0.49 hrs and 37.4 hours for α, β and γ phases of the kinetics. For dog J, the observed $t_{1/2}$ values were 21.2 min, 1.26 hrs and 16.3 hrs. The average plasma concentrations at various times are shown in FIG. 7.

Urine specimens were collected from each dog at 30 min, 1 hr, 2 hr and 4 hr following administration of 10-propargyl-10dAM and analyzed by HPLC. 10-propargyl-10dAM was mainly excreted unchanged (retention time 18.5 minutes). There were small amounts of a metabolite with a retention time of 6.3 min which account for <0.31% and <3.5% of the total urinary 10-propargyl-10dAM at 1 hr and 4 hr, respectively.

What is claimed is:

1. 10-Propargyl-10-deazaaminopterin, substantially free of 10-deazaaminopterin.

2. A composition consisting essentially of 10-Propargyl-10-deazaaminopterin.

3. A pharmaceutical composition comprising 10-Propargyl-10-deazaaminopterin, substantially free of 10-deazaaminopterin, and a pharmaceutically acceptable carrier.

4. A method for treatment of tumors comprising administering to a human patient diagnosed as having a tumor a therapeutically effective amount of 10-propargyl-10-deazaaminopterin, substantially free of 10-deazaaminopterin.

5. The method according to claim **4**, wherein the tumor is a solid tumor.

6. The method according to claim **4**, wherein the 10-propargyl-10-deazaaminopterin, substantially free of 10-deazaaminopterin, is administered in amounts of from 40 to 120 mg/m^2 of body surface area/day.

7. The method according to claim **5**, wherein the tumor is a mammary tumor.

8. The method according to claim **4**, wherein the tumor is a lung tumor.

9. The pharmaceutical composition according to claim **3**, further comprising at least one additional cytotoxic or antitumor compound.

10. The pharmaceutical composition according to claim **9**, wherein the at least one additional cytotoxic or antitumor compound is selected from the group consisting of vinca alkaloids, 5-fluorouracil, alkylating agents, cisplatin, carboplatin, leucovorin, taxols and antibiotics.

11. The method according to claim **4**, wherein at least one additional cytotoxic or antitumor compound is administered with the therapeutically effective amount of 10-propargyl-1-deazaaminopterin, substantially free of 10-deazaaminopterin.

12. The method according to claim **11**, wherein the at least one additional cytotoxic or antitumor compound is selected from the group consisting of vinca alkaloids, 5-fluorouracil, alkylating agents, cisplatin, carboplatin, leucovorin, taxols and antibiotics.

* * * * *

EXHIBIT B

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Customer No 52334

ISTMT

DATE PRINTED
10/13/2009

Larson & Anderson, LLC
re: MSK
P. O. BOX 4928
DILLON CO 80435-4928

MAINTENANCE FEE STATEMENT

According to the records of the U.S.Patent and Trademark Office (USPTO), the maintenance fee and any necessary surcharge have been timely paid for the patent listed below. The "PYMT DATE" column indicates the payment date (i.e., the date the payment was filed).

The payment shown below is subject to actual collection. If the payment is refused or charged back by a financial institution, the payment will be void and the maintenance fee and any necessary surcharge unpaid.

Direct any questions about this statement to: Mail Stop M Correspondence, Director of the USPTO, P.O.Box 1450, Alexandria, VA 22313-1450.

PATENT NUMBER	FEE AMT	SUR CHARGE	PYMT DATE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	ATTY DKT NUMBER
6,028,071	**$445.00**	**$0.00**	**07/15/03**	**09/214,984**	**02/22/00**	**03/08/99**	**04**	**YES**	**MSK.P-003-US/NP**

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Customer No 52334

ISTMT

DATE PRINTED
10/13/2009

Larson & Anderson, LLC
re: MSK
P. O. BOX 4928
DILLON CO 80435-4928

MAINTENANCE FEE STATEMENT

According to the records of the U.S.Patent and Trademark Office (USPTO), the maintenance fee and any necessary surcharge have been timely paid for the patent listed below. The "PYMT DATE" column indicates the payment date (i.e., the date the payment was filed).

The payment shown below is subject to actual collection. If the payment is refused or charged back by a financial institution, the payment will be void and the maintenance fee and any necessary surcharge unpaid.

Direct any questions about this statement to: Mail Stop M Correspondence, Director of the USPTO, P.O.Box 1450, Alexandria, VA 22313-1450.

PATENT NUMBER	FEE AMT	SUR CHARGE	PYMT DATE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	ATTY DKT NUMBER
6,028,071	**$1,150.00**	**$0.00**	**07/16/07**	**09/214,984**	**02/22/00**	**03/08/99**	**08**	**YES**	**MSK.P-003**

EXHIBIT C

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,028,071
APPLICATION NO. : 09/214984
DATED : February 22, 2000
INVENTOR(S) : Sirotnak et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 1, Line 9 please insert: This application was supported by NIH grant number CA56517. The US government has certain rights in this invention.

Signed and Sealed this

Eighth Day of April, 2008

JON W. DUDAS
Director of the United States Patent and Trademark Office